Patterns of Heteroduplex Formation Associated With the Initiation of Meiotic Recombination in the Yeast *Saccharomyces cerevisiae*

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Manuscript received January 29, 2003
Accepted for publication May 6, 2003

ABSTRACT

The double-strand break repair (DSBR) model of recombination predicts that heteroduplexes will be formed in regions that flank the double-strand break (DSB) site and that the resulting intermediate is resolved to generate either crossovers or noncrossovers for flanking markers. Previous studies in *Saccharomyces cerevisiae*, however, failed to detect heteroduplexes on both sides of the DSB site. Recent physical studies suggest that some recombination events involve heterodupex formation by a mechanism, synthesis-dependent strand annealing (SDSA), that is inherently asymmetric with respect to the DSB site and that leads exclusively to noncrossovers of flanking markers. Below, we demonstrate that many of the recombination events initiated at the *HIS4* recombination hotspot are consistent with a variant of the DSBR model in which the extent of heteroduplex on one side of the DSB site is much greater than that on the other. Events that include only one flanking marker in the heteroduplex (unidirectional events) are usually resolved as noncrossovers, whereas events that include both flanking markers (bidirectional events) are usually resolved as crossovers. The unidirectional events may represent SDSA, consistent with the conclusions of others, although other possibilities are not excluded. We also show that the level of recombination reflects the integration of events initiated at several different DSB sites, and we identify a subset of gene conversion events that may involve break-induced replication (BIR) or repair of a double-stranded DNA gap.

MEIOTIC recombination events in the yeast *Saccharomyces cerevisiae* are initiated by double-stranded DNA breaks (Szostak *et al.* 1983; Sun *et al.* 1989) catalyzed by the protein Spo11 (Bergerat *et al.* 1997; Keeney *et al.* 1997). Until recently, in the most widely accepted version of the double-strand break repair (DSBR) model, the DNA ends resulting from the break are resected 5′ to 3′, and one of the resected "tails" invades the homologous chromatid, forming a region of heteroduplex (Figure 1). DNA synthesis primed from this 3′ end results in a second heteroduplex, involving the second tail. The two regions of heteroduplex, one on each chromatid, are flanked by two Holliday junctions. Cleavage of these junctions can result in the flanking chromosomal regions in either crossover or noncrossover configurations. In this model, both crossovers and noncrossovers have the same molecular precursor.

In Figure 1, we illustrate a recombination event initiated between two heterozygous genes with alleles *A* and *a* and *B* and *b*. If the heteroduplexes include the region of the gene with the mutation (as shown), then two DNA mismatches on two different chromatids would be formed. If these mismatches are not repaired, one of the four spores will have a postmeiotic segregation (PMS) event at the *A* locus, and a different spore in the same tetrad would have a PMS event at the *B* locus; PMS events are defined as the segregation of two alleles from a single meiotic product at the first mitotic division following meiosis (Petes *et al.* 1991). For some loci, these events can be detected by replica plating the spore colonies to diagnostic omission medium. Alternatively, one can detect such events using the polymerase chain reaction (PCR; Porter *et al.* 1993). Repair of the mismatches will result in gene conversion tetrads, tetrads that have either three wild-type spores and one mutant spore or one wild-type spore and three mutant spores (Petes *et al.* 1991). For example, repair of the mismatch shown in Figure 1 could result in a tetrad with 3*A*:1*a* segregation pattern.

Although both physical and genetic evidence supports the existence of several of the intermediates shown in Figure 1 [for example, the double Holliday junction (Schwacha and Kleckner 1995)], there are also genetic and physical data that support the argument that all recombination events do not occur through the canonical form of the DSBR model. The genetic experiments used strains that were heterozygous for markers closely flanking a recombination hotspot (a site of frequent meiosis-specific DSB formation). These markers were designed to yield DNA mismatches that were inef-

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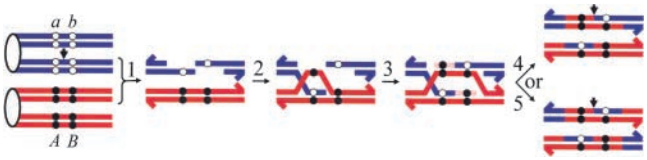

FIGURE 1.—Canonical DSBR model (SZOSTAK *et al.* 1983; SUN *et al.* 1991). In this diagram, the DSB (indicated by the vertical arrow) occurs between two heterozygous markers on one of the chromatids with the mutant *a* and *b* alleles. The solid and open circles represent wild-type and mutant substitutions, respectively. The broken ends are processed 5′ to 3′, leaving protruding 3′ ends (step 1). Step 2 shows the left end of the broken chromatid invading one of the unbroken chromatids (forming a heteroduplex with a mismatch). In step 3, DNA synthesis occurs (represented by a stippled red strand), resulting in displacement of the red strand. The displaced strand pairs with the broken right end, resulting in a second region of heteroduplex with a mismatch. DNA synthesis primed from the 3′ end of the blue chromatid also occurs. Steps 4 and 5 represent alternative ways of cutting the strands connecting the two chromatids, resulting in chromatids without (step 4) or with (step 5) an associated crossover. Failure to correct the mismatches would result in a tetrad that had 5:3 segregation for both flanking markers. Correction of the mismatch would generate a 3:1 segregation event, if the correction were in the direction of the wild-type substitution, or would restore 2:2 segregation, if the correction was in the direction of the mutant substitution.

ficiently repaired if involved in heteroduplex formation, leading primarily to PMS tetrads. As discussed above, the DSBR model predicts a high frequency of tetrads in which one spore has a PMS event for one of the flanking markers and another spore in the same tetrad has a PMS event for the other marker. In two studies involving different loci, coevents of this type were very rare (PORTER *et al.* 1993; GILBERTSON and STAHL 1996), although events involving only one of the two markers were very common (unidirectional events).

ALLERS and LICHTEN (2001a,b) found physical data in conflict with the canonical DSBR model. In an ectopic recombination system, they showed that noncrossover heteroduplex products were completed before crossover heteroduplex products. Furthermore, they found that a mutation of *NDT80*, a meiosis-specific transcription factor, resulted in normal levels of noncrossover heteroduplexes, but greatly reduced crossovers. On the basis of these results, Allers and Lichten proposed that the heteroduplex intermediates for crossovers and noncrossovers were different: crossovers involving resolution of intermediates as shown in Figure 1 and noncrossovers occurring by a different pathway [synthesis-dependent strand annealing (SDSA)]. The SDSA pathway was originally suggested as a way of explaining mitotic gene conversion events that were unassociated with crossing over (reviewed by PAQUES and HABER 1999). In this model, following initial strand invasion and repair synthesis, the invading strand containing newly synthesized DNA is displaced and reannealed to the other 3′ end. The net result of this event is heteroduplex formation on one side of the DSB in one chromatid with a noncrossover configuration of the flanking sequences.

In our previous study of heteroduplex formation (PORTER *et al.* 1993), we used heterozygous markers that were ~1 kb from the *HIS4* hotspot DSB site. Below, we describe experiments performed with a strain that has markers <250 bp from this DSB site and includes markers located ~5 kb from the site. Using this new system, we identified bidirectional events with the configuration of heteroduplex predicted by the DSBR model. These events are primarily crossovers, but a significant fraction are noncrossovers. We also observed unidirectional events similar to those previously observed; most, but not all, of these events represent noncrossovers. We interpret these data as indicating that recombination in *S. cerevisiae* proceeds by both the canonical DSBR and the SDSA pathways.

MATERIALS AND METHODS

Yeast strains: All strains used were derived by transformation from the haploid strains AS4 (α *trp1 arg4 tyr7 ade6 ura3*) and AS13 (*a leu2 ura3 ade6*; STAPLETON and PETES 1991). In the descriptions of the genotypes, we note only deviations from the two parental genotypes.

We constructed JDM173 (*fus1-BX*) by two-step transplacement of AS4 using *Kpn*I-digested pMW30 (WHITE and PETES 1994). All constructions, unless noted otherwise, were checked by PCR. JDM179 (*bik1-lop his4u-lopc ycl034W-SX*) was constructed in two steps. First, PD57 (DETLOFF *et al.* 1992), an AS13 derivative with a deletion of a portion of the *HIS4* upstream activating sequences (UAS), was transformed with a DNA fragment containing the wild-type UAS of *HIS4* and two palindromic insertions (*bik1-lop* and *his4u-lopc*) to generate JDM148 (*bik1-lop his4u-lop*). This DNA fragment was generated using PCR of genomic DNA derived from AS13 cells and the primers (with the palindromic sequences underlined) his4u + lopF1 (5′-GATTCCTCATCGGAAGAGGTGGCATCCTTAACGAAAA AACGAGTACTGTATGTACATACAGTACTCTTGAAGAGGC TAATGAAAAA) and his4u + lopcR1(5′-AGTTGTGCTATGA TATTTTTATGTATGTACAACACACATCGACTAGTCTAAG TACTTAGACTAGTCGGAGGTGAATATAACGTTCC). Correct transplacement of the PCR fragment results in restoration of histidine prototrophy, and histidine prototrophs were sequenced to confirm the construction. Second, the *ycl034W-SX* mutation was inserted into JDM148 using a two-step transplacement of *Sna*BI-digested pJDM4 (described below) to generate JDM179.

The plasmid pJDM4 was derived from pMW25, a plasmid with a *Bgl*II fragment containing *YCL034W* from pC1G-17 (PORTER *et al.* 1993) inserted into the *Bam*HI site of YIp5 (STRUHL *et al.* 1979). pJDM4 was made by "filling in" (SYMINGTON and PETES 1988) the *Spe*I site in *YCL034W*, resulting in the allele *ycl034W-SX*.

MD229 (*bik1-lop his4u-lopc his4-IR9 ycl034W-SX*) was constructed by inserting the *his4-IR9* mutation into JDM179 using a two-step transplacement of *Sna*BI-digested pDN22 (NAG and PETES 1991). MD248 (*fus1-BX cha1::hphMX4*) and MD249 (*bik1-lop his4u-lopc his4-IR9 ycl034W-SX cha1::hphMX4*) were constructed by inserting the *hphMX4* cassette (GOLDSTEIN and MCCUSKER 1999), which contains a gene that confers hygromycin resistance, into the middle of *CHA1* in JDM173 and MD229, respectively. The PCR synthesis of the cassette and

subsequent transformation were performed as described by Wach *et al.* (1994), using the plasmid pAG32 (Goldstein and McCusker 1999) and the primers CHA-F (5′-TCCC TTCGATAATCCGGATATTTGGGAAGGACATTCATCTATG ATAGATGCGTACGCTGCAGGTCGAC) and CHA-R (5′-TTT AACCTTATTCACGGAAATATGTTGCGATTTCAAATCTTG TACTATTTATCGATGAATTCGAGCTCG).

PG118 (*fus1-BX rad50S*) and PG119 (*bik1-lop his4u-lopc ycl034W-SX rad50S*) are *rad50S* derivatives of JDM173 and JDM179, respectively, and were constructed as described by Alani *et al.* (1990). PG138 (*his4-IR9 ycl034W-SX*) was constructed by inserting the *ycl034W-SX* mutation into DNY47 (Nag and Petes 1991), using pJDM4 as described above.

We made the diploid strains by mating the following haploid strains (given in parentheses after the name of the diploid): JDM1080 (JDM173 × JDM179), JDM1081 (PG118 × PG119), JDM1086 (JDM173 × MD229), JDM1091 (JDM173 × PG138), MD250 (MD248 × MD229), MD251 (JDM173 × MD249), and QF105 (Fan *et al.* 1995). The genotypes of these strains (not including the AS4- and AS13-derived markers common to all of the strains) are: JDM1080 (*FUS1/fus1-BX bik1-lop/BIK1 his4u-lopc/HIS4U ycl034W-SX/YCL034W*), JDM1081 (*FUS1/fus1-BX bik1-lop/BIK1 his4u-lopc/HIS4U ycl034W-SX/YCL034W rad50S/rad50S*), JDM1086 (*FUS1/fus1-BX bik1-lop/BIK1 his4u-lopc/HIS4U his4-IR9/HIS4 ycl034W-SX/YCL034W*), JDM1091 (*FUS1/fus1-BX his4-IR9/HIS4 ycl034W-SX/YCL034W*), MD250 (*FUS1/fus1-BX bik1-lop/BIK1 his4u-lopc/HIS4U his4-IR9/HIS4 ycl034W-SX/YCL034W CHA1/cha1::hphMX4*), MD251 (*FUS1/fus1-BX bik1-lop/BIK1 his4u-lopc/HIS4U his4-IR9/HIS4 ycl034W-SX/YCL034W cha1::hphMX4/CHA1*), and QF105 (*his4-IR9/HIS4 rad50S/rad50S*). The designation *HIS4U* signifies that the *HIS4* promoter region is wild type.

Genetic analysis: Standard materials and methods were used (Sherman *et al.* 1983) except where noted. Diploids were sporulated on plates at 25°, rather than 18° (the temperature used for most of our previous studies), to reduce the frequency of multiple recombination events at the *HIS4* hotspot (Nag *et al.* 1989). Following tetrad dissection on plates with rich growth medium (YPD), the spore colonies were replica plated to various omission media; spore colonies on medium lacking histidine were examined microscopically to detect small sectors.

Experiments to determine whether the transcribed or nontranscribed strand of *HIS4* was transferred were performed as described by Nag and Petes (1990) with the following modifications. Tetrads were dissected onto plates containing histidine omission medium (SD − his) and incubated at 30° for 10–12 hr. The spores were then examined microscopically and scored as His^+ (5 or more cells) or His^- (1 or 2 cells). The SD − his agar slab containing the spores was transferred to a YPD plate with 0.4 ml of a 0.5% histidine solution. After incubation at 30° for 3–4 days, the colonies were replica plated to SD − his medium (as well as other omission media) to determine the pattern of sectoring. Only tetrads with a 5:3 or 3:5 pattern of aberrant segregation were scored for the remaining markers by PCR. The pattern of sectoring was then correlated with the phenotype of the spore to determine whether the transcribed or nontranscribed strand of *HIS4* had been donated (Nag and Petes 1990). This analysis allows us to determine whether the DSB in a given recombination event occurred upstream (nontranscribed strand donated) or downstream (transcribed strand donated) of our markers in *HIS4*.

PCR analysis of spore colonies: We performed PCR analysis to score the *fus1-BX*, *bik1-lop*, *his4u-lopc*, and *ycl034W-SX* markers. All PCRs were performed in 96-well trays using the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). PCR conditions were those suggested by the manufacturer of AmpliTaq polymerase (Applied Biosystems) with the following modifications. The reaction conditions contained 1.5 mm $MgCl_2$ for the PCR to score *bik1-lop* and 2.5 mm $MgCl_2$ for all other PCRs. All four dNTPs were added to a final concentration of 400 μm for the PCR to score *fus1-BX* and *ycl034W-SX* and 200 μm for the other PCRs. All PCRs contained 0.8 μm of each primer. The reactions used to score *fus1-BX* and *ycl034W-SX* contained 2.5 units of AmpliTaq, and all other reactions contained 1.75 units of AmpliTaq.

A toothpick was used to mix the cells of the spore colony on a YPD replica of the dissection plate. The cells were then transferred to 6 μl of sterile, distilled water in the 96-well tray. Each tray included three control reactions where the cells were wild type, mutant, or sectored for the marker of interest. The samples were heated to 94° for 6 min and subsequently placed at −80° for 10 min. The samples were thawed at room temperature, and the remaining 19 μl of the reaction components was added. Samples were exposed to the following conditions for 40 cycles: 94° for 1 min, 57° for 1 min, and 72° for 3 min.

The primers BIK1 + 1021F (5′-ACGATTCGCTCAGTAAA GAATAC) and BIK1 + 1228R (5′-GCCGTGGTATCGACTGG TGC) produce a 208-bp product if the wild-type sequence is present and a 234-bp product if the *bik1-lop* sequence is present. The PCR products were digested with *Bsr*GI (New England Biolabs, Beverly, MA), which cuts within the *bik1-lop* sequence. Subsequently, the digested PCRs were resolved on a 3.5% MetaPhor agarose (BioWhittaker Molecular Applications, Walkersville, MD) gel. Three patterns of bands were observed: a 208-bp fragment if only the wild-type *BIK1* sequence was present in the spore colony, a pair of 115- and 119-bp fragments if only the *bik1-lop* sequence was present in the spore colony, and a set of 115-, 119-, 208-, and apparently 240-bp fragments if both wild-type and *bik1-lop* sequences were present in the spore colony (a PMS event). The fragment with the apparent size of 240 bp is likely an *in vitro*-generated heteroduplex fragment containing one wild-type and one *bik1-lop* strand.

The primers HIS4-210F (5′-CCCATGCACAGTGACTCACG) and HIS4 + 42R (5′-ATGAGGCCAGATCATCAATTAACGG) produce a 253-bp product if the wild-type sequence is present and a 279-bp product if the *his4u-lopc* sequence is present. The PCR products were digested with *Sca*I (New England Biolabs), which cuts within *his4u-lopc*. Upon gel analysis, three patterns (similar to those observed for *bik1-lop*) were seen: a 253-bp fragment if only the wild-type *HIS4* sequence was present in the spore colony, a pair of 134- and 145-bp fragments if only the *his4u-lopc* sequence was present in the spore colony, and a set of 134-, 145-, 253-, and apparently 300-bp fragments if both wild-type and *his4u-lopc* sequences were present in the spore colony. Using this method to score *bik1-lop* or *his4u-lopc*, we always (10 of 10 times) detected mutant or wild-type sequences, even when they represented <10% of the DNA sample.

For most of the spores that had PMS for more than one marker, we determined whether the configuration of the markers was *in cis* (palindromes on the same DNA strand in the heteroduplex) or *trans* (palindromes on different DNA strands). Cells from the relevant spore colony were streaked onto YPD. When single colonies had formed, two independent colonies were examined by PCR or replica plating (as described above) for the relevant markers.

We scored both flanking markers, *fus1-BX* and *ycl034W-SX*, using a single PCR. The PCRs were digested simultaneously with *Spe*I and *Bcl*I (New England Biolabs) and resolved on 1.5% agarose gels. The primers SpeI-508F (5′-ACGCTAGAAG TGGAGTTAGC) and SpeI + 276R (5′-AACGCAGCCACCAGT TCATC) produce a fragment of ~800 bp. A fragment containing wild-type sequence (*YCL034W*) produces fragments of

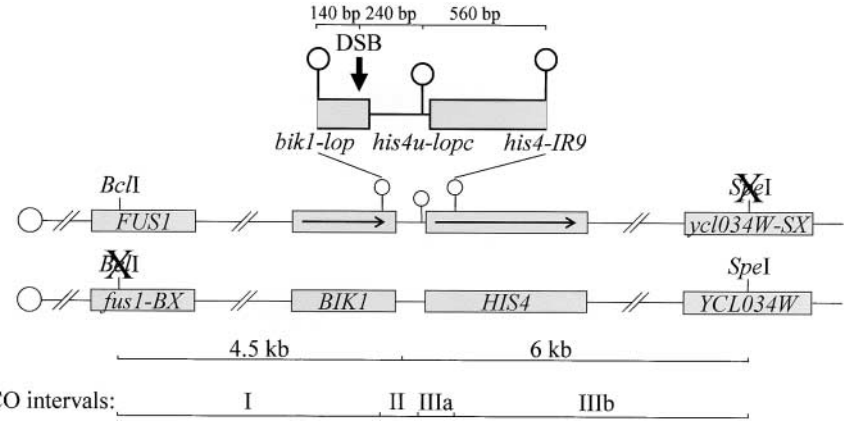

FIGURE 2.—Arrangement of genetic markers in strains JDM1080 and JDM1086. The position of the DSB associated with the *HIS4* recombination hotspot is indicated by the arrow. The "lollipops" indicate short palindromic insertions, markers that result in inefficiently repaired mismatches. JDM-1080 lacks the *his4-IR9* marker; otherwise, the strains are identical.

~300 and 500 bp when digested with *Spe*I, while a fragment with the *Spe*I "fill in" (*ycl034W-SX*) remains 800 bp. The primers FUS1 + 517(I)F (5′-CCGCAGCATATACTGACACC) and FUS1 + 1514(I)R (5′-AGTCACCAGGCACAATGCCT) produce a fragment of ~1 kb. A fragment containing wild-type sequence (*FUS1*) produces fragments of ~400 and 600 bp when digested with *Bcl*I, while a fragment with the *fus1-BX* mutation remains 1 kb. The *fus1-BX* and *ycl034W-SX* markers generally did not exhibit sectoring, which is typical for 4-bp insertions (NAG *et al.* 1989).

Southern analysis: Cells were harvested from *rad50S* diploid strains just prior to being placed on a sporulation plate (0 hr) or after 24 hr on sporulation medium. Cells were washed with 0.5 ml 10 mM Tris (pH 8.0), 1 mM EDTA, and stored at −80°. DNA isolation and Southern blot procedures were performed as described by NAG and PETES (1993).

This procedure was used to map DSBs occurring in a 15-kb interval centered on *HIS4*. Probes were prepared from genomic DNA by PCR; 20-bp primers were derived from the sequence intervals described below. The *HIS4-BIK1* region [*Saccharomyces* Genome Database (http://genome-www.stanford.edu/Saccharomyces/) chromosome III coordinates 66,644–69,621] was examined using a *Bgl*II digest and a *Bgl*II-*Xho*I fragment of *HIS4* as a hybridization probe (NAG and PETES 1993). The *YCL036W-YCL031C* region (60,396–65,125) was examined with an *Sph*I digest and a hybridization probe covering the coordinates 60,410–61,152. The *YCL031C-HIS4* region (64,542–68,093) was examined with an *Nhe*I digest and a hybridization probe covering coordinates 66,175–67,276. The *BIK1-FUS1* region (69,019–73,467) was examined using a *Ban*I digest and a hybridization probe covering coordinates 69,073–69,817. The *FUS1-YCL025C* region (72,688–77,018) was examined using an *Nci*I digest and a hybridization probe covering coordinates 72,768–73,341. Hybridization was quantitated using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA), and the percentage of molecules with a DSB was calculated as described (KIRKPATRICK *et al.* 1999). All levels of DSBs were normalized to the DSB associated with the *HIS4* hotspot.

Data analysis: Statistical analysis was performed using Instat 1.12 (GraphPad Software) for the Macintosh. The Fisher's exact test with a two-tailed P value or chi-square analysis (for comparisons that involve more than two experimental measures) was used for all comparisons, and $P < 0.05$ was considered statistically significant.

RESULTS

Experimental system: We designed related diploid strains, JDM1086 and JDM1080 (Figure 2), to examine the arrangement of heteroduplex DNA close to the *HIS4* DSB site and the association of heteroduplex DNA with a crossover or noncrossover configuration of flanking sequences. Both strains were heterozygous for short palindromic insertions (*bik1-lop* and *his4u-lopc*) closely flanking the DSB site, and JDM1086 was also heterozygous for *his4-IR9*, a short palindromic insertion within the *HIS4* coding sequence. The strains were sporulated and tetrads derived from the strains were dissected. A mismatch resulting from a heteroduplex with one wild-type DNA strand and one strand with a short palindromic insertion is inefficiently repaired to generate a gene conversion, resulting in frequent PMS events (NAG *et al.* 1989). For the *bik1-lop* and *his4u-lopc* markers, such events can be detected by PCR (details in MATERIALS AND METHODS); a PMS event involving *his4-IR9* can be detected by replica plating the spore colony to medium lacking histidine. The segregation of the flanking heterozygous restriction site markers *fus1-BX* and *ycl034W-SX* was also analyzed by PCR.

A summary of the segregation of markers in the two strains is in Table 1. Since (as described in the Introduction) heteroduplex formation can result in spores that have genes in which one DNA strand has wild-type and one strand has mutant information, it is convenient to describe the patterns of aberrant segregation using the nomenclature derived from eight-spored fungi. We classify gene conversion tetrads with three wild-type and one mutant or one wild-type and three mutant spore colonies as 6:2 and 2:6, respectively. Tetrads with two wild-type, one mutant, and one wild-type/mutant PMS spore colonies and those with one wild-type, two mutant, and one wild-type/mutant PMS spore colonies were classified as 5:3 and 3:5, respectively. Aberrant 4:4 tetrads have one wild-type, one mutant, and two wild-type/mutant PMS spore colonies. Several different methods of analysis were done in the JDM1086 strain. For the data designated "unselected," we examined the segregation of all markers in every spore colony in tetrads with four viable spores. The other methods of analysis are discussed further below.

Unselected tetrads of JDM1086 and JDM1080 had

TABLE 1

Aberrant segregation and *HIS4/LEU2* map distances for strains used in this study

Strain	Method of analysis	Total tetrads	Mutant allele	6:2	2:6	5:3	3:5	0:4	Aberrant 4:4	Other PMS	ABS (%)	PMS/ABS (%)	*HIS4/LEU2* map distance[a](cM)
JDM1080	Unselected	59	*his4u-lopc*	3	2	6	4	0	1	0	27	69	24
			bik1-lop	3	1	6	3	0	1	0	24	71	
			fus1-BX	5	2	0	0	0	0	0	12	0	
			ycl034W-SX	2	8	0	0	0	0	0	17	0	
JDM1086	Unselected	57	*his4-IR9*	1	0	4	6	0	0	0	19	90	21
			his4u-lopc	2	2	3	9	0	0	0	28	75	19
			bik1-lop	2	0	3	11	0	1	1[b]	32	90	
			fus1-BX	4	4	0	0	0	0	0	14	0	
			ycl034W-SX	2	5	0	0	0	0	0	12	0	
JDM1086	Selected-1[c]	712	*his4-IR9*	22	23	37	41	0	6	2[d]	18	66	24
JDM1086	Selected-2[e]	594	*his4-IR9*	22	10	38	32	1	3	2[f]	18	69	24
JDM1086	Strand transfer	181	*his4-IR9*	8	3	11	14	0	0	1[g]	20	70	21
JDM1091	Unselected	158	*his4-IR9*	6	6	12	13	0	0	0	23	68	20
		104	*fus1-BX*	7	9	0	0	0	0	0	15	0	
		104	*ycl034W-SX*	6	11	0	0	0	0	0	16	0	
MD250	Selected-LCT[h]	302	*his4-IR9*	9	12	14	18	0	0	2[i]	18	62	21
			cha1::hphMX4	4	4	0	0	0	0	0	3	0	
MD251	Selected-LCT[h]	1127	*his4-IR9*	46	49	70	86	0	4	5[j]	23	63	21
			cha1::hphMX4	15	7	1	1	0	0	0	2	8	

ABS, total aberrant segregations; PMS, postmeiotic segregations.

[a] Calculated using the Perkins (1949) equation. The *HIS4/LEU2* map distance for MD251 was based on the analysis of 429 tetrads.

[b] One aberrant 2:6.

[c] After scoring of the *his4-IR9* marker, only those events exhibiting a 6:2, 2:6, 5:3, or 3:5 pattern of aberrant segregation (single events) at *his4-IR9* were scored for the remainder of the markers.

[d] One aberrant 6:2 and one 7:1.

[e] This selection method was designed to identify tetrads that underwent a single recombination event initiated at the *HIS4* DSB. First, *his4-IR9* was scored for all tetrads. Next, all spores exhibiting a single PMS event at *his4-IR9* were scored for *bik1-lop*. Only those tetrads in which the spore did not cosector for *his4-IR9* and *bik1-lop* were completely scored for *bik1-lop*. Tetrads consistent with a single recombination event being initiated at *HIS4* were scored for *his4u-lopc* and subsequently the flanking markers (*fus1-BX* and *ycl034W-SX*).

[f] One aberrant 2:6 and one 1:7.

[g] One 7:1.

[h] This method was used to identify events with long conversion tracts (LCT), events that involved conversion of all markers in the same direction (for example, 6:2 for *fus1-BX* and 2:6 for *bik1-lop, his4u-lopc, his4-IR9*, and *ycl034W-SX*). All tetrads were scored for *his4-IR9* and *cha1::hphMX4*. Those events that involved conversion of both markers were subsequently scored for the flanking markers, *bik1-lop*, and *his4u-lopc* (if the markers continued to show conversion in the same direction).

[i] Two 7:1.

[j] One aberrant 2:6, three 7:1, and one 1:7.

similar frequencies of aberrant segregation for the same markers. The frequencies of aberrant segregation of the *fus1-BX*, *his4-IR9*, and *ycl034W-SX* markers in JDM1086 were similar to those observed in strain JDM1091. JDM-1091 is identical to JDM1086 except that it lacks the *bik1-lop* and *his4u-lopc* markers. The frequencies of crossovers between *HIS4* and the linked *LEU2* gene in the two strains were also very similar (Table 1). If a mutation generates a recombination hotspot in a strain heterozygous for the mutation, one finds an excess of tetrads of the 6:2 and 5:3 classes over the 2:6 and 3:5 classes (Petes *et al.* 1991). No such disparity is observed for the palindromic markers in JDM1086 or JDM1080 (Table 1), indicating that these palindromes neither stimulate nor repress recombination. We confirmed this result by mapping DSBs associated with the *HIS4* hotspot in the strains JDM1081 (a *rad50S* derivative of JDM1080) and QF105 (a *rad50S* strain without the *his4u-lopc* and *bik1-lop* markers). The positions and levels of DSBs in the two strains were the same (data not shown).

In addition to examining unselected tetrads in JDM-1080 and JDM1086, we used several other methods of analysis for JDM1086. For tetrad data classified as "selected-1" (S1), we screened for tetrads in which the *his4-IR9* marker (which can be scored by replica plating to medium lacking histidine) segregated 6:2, 2:6, 5:3, or 3:5 (indicative of a single recombination event involving the marker), and we subsequently examined the other markers (*fus1-BX*, *bik1-lop*, *his4u-lopc*, and *ycl034W-SX*) by PCR. For tetrad data classified "selected-2" (S2), we screened for tetrads that segregated 5:3 or 3:5 at the *his4-IR9* locus and did not have a cosector for the *bik1-lopc* marker in the same spore. If the pattern was consistent with a single event initiated at the *HIS4* hotspot, we examined all of the other markers. As described below, this procedure selects against tetrads in which the recombination event is initiated at a DSB that is different from the *HIS4* hotspot DSB. The "strand transfer" method of analysis is described below.

Classification of recombination events: As in previous experiments involving multiple markers located near a recombination hotspot (for example, Porter *et al.* 1993), we find many classes of tetrads. All the data are on the website (http://www.genetics.org/supplemental/). The data are divided into five supplementary tables: Table I (single unidirectional recombination events initiated at the *HIS4* hotspot), Table II (single bidirectional recombination events initiated at the *HIS4* hotspot), Table III (single recombination events initiated at a site other than the *HIS4* hotspot), Table IV (unambiguous multiple recombination events), and Table V (events in which the classification as single or multiple events is model dependent).

The tetrads that are most useful in exploring the nature of DSB-mediated recombination are those with a single initiating DNA lesion occurring at the *HIS4* hotspot (between the *bik1-lop* and *his4u-lopc* markers). Determination of where the events were initiated and whether the recombination events were initiated by one or multiple DSBs was based on several assumptions. First, all recombination events are initiated by a DSB, occurring at the *HIS4* hotspot or one of the other hotspots mapped (as described below) within an 11-kb region that includes the *HIS4* hotspot. Second, recombination events involve the continuous asymmetric transfer of a single strand from one chromosome to another, resulting in the formation of heteroduplex on one side of the DSB in one chromatid, but (potentially) heteroduplex formation on the other side of the DSB in a different chromatid (Figure 1). Third, the initiating DSB occurs at one end of a heteroduplex tract. If an event is unidirectional and both ends of the heteroduplex tract correspond to meiotic DSB sites, the initiating DSB is assumed to occur at the stronger DSB site. If an event is bidirectional (involves two regions of heteroduplex on different chromatids), the DSB is assumed to occur between the two regions of heteroduplex. Fourth, Holliday junctions are resolved at the ends of the heteroduplex tracts. This last assumption will lead to some degree of underestimation of the frequency of associated crossovers, since a mismatch repair event that leads to restoration of Mendelian segregation (reviewed in Petes *et al.* 1991) would separate the detectable heteroduplex tract from the position of the crossover. We note, however, that the three palindromic insertions occur near the *HIS4* DSB site, where restoration repair occurs infrequently (Detloff *et al.* 1992).

Of 1603 tetrads derived from strains JDM1086 and JDM1080, there were 217 tetrads with an aberrant segregation event for one or more of the five heterozygous markers in the *HIS4* region; 56 are explicable as resulting from a single DSB located at the *HIS4* hotspot and 77 are explicable as resulting from a single DSB located at a site other than the *HIS4* hotspot. In addition, 50 tetrads had undergone multiple initiation events, and 34 can be classified as either single- or multiple-event tetrads, depending on details of the models of recombination.

Single recombination events initiated at the *HIS4* DSB: The tetrads that we classify as single recombination events initiated at the *HIS4* DSB share several properties: (1) tracts of aberrant segregation are uninterrupted by markers undergoing normal Mendelian segregation, (2) markers on each side of the DSB site have aberrant segregation properties indicating involvement of a single donated DNA strand, and (3) markers on opposite sides of the DSB site involve different chromatids. In 116 unselected tetrads of JDM1086 and JDM1080, 22 (19%) had these properties. Of all tetrads examined for these strains, we classified 56 as representing single events initiated at the *HIS4* hotspot.

A total of 70% of these tetrads (40 of 56) were classified as unidirectional events (Table I, classes 1–25). Unidirectional events exhibit continuous tracts of aber-

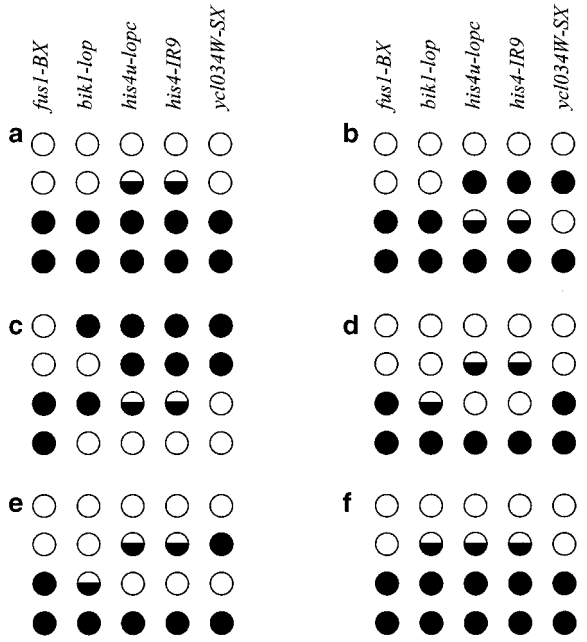

FIGURE 3.—Examples of segregation patterns representing different types of recombination. We illustrate segregation patterns for five markers heterozygous in JDM1086. As in Tables I–V at http://www.genetics.org/supplemental/, each row of circles represents a spore colony. For the markers *bik1-lop*, *his4u-lopc*, *his4-IR9*, and *ycl034W-SX*, solid circles represent colonies with the wild-type genotype and open circles represent the mutant genotype. For *fus1-BX*, solid and open circles represent mutant and wild-type genotypes, respectively. Thus, a tetrad with two rows of open circles and two rows of solid circles would indicate a nonrecombinant tetrad. Solid/open sectored circles represent PMS events. The class numbers indicated below are those used in Tables I–V, available at http://www.genetics.org/supplemental/. (a) Tetrad with unidirectional event initiated at the *HIS4* hotspot unassociated with crossover (Table I, class 12). (b) Tetrad with unidirectional event initiated at the *HIS4* hotspot associated with crossover between *bik1-lop* and *his4u-lopc* (Table I, class 13). (c) Tetrad with unidirectional event initiated at the *HIS4* hotspot associated with crossover between *bik1-lop* and *his4u-lopc* and an incidental crossover involving two other chromatids (Table IV, class 111). (d) Tetrad with bidirectional event initiated at the *HIS4* hotspot unassociated with crossover (Table II, class 30). (e) Tetrad with bidirectional event initiated at the *HIS4* hotspot associated with crossover either between *fus1-BX* and *bik1-lop* or between *his4-IR9* and *ycl034W-SX* (Table II, class 31). (f) Tetrad with single recombination event initiated at a DSB other than the *HIS4* hotspot (Table III, class 64).

rant segregation on one side of the DSB (toward either *HIS4* or *BIK1*) that are confined to a single chromatid. About 30% (10 of 34 tetrads in which the configuration of the flanking sequences could be unambiguously assigned) of these events are associated with crossovers. Examples of segregation patterns consistent with unidirectional events without and with associated crossovers are shown in Figure 3, a and b, respectively.

This number of undirectional events with associated crossovers is larger than can be explained by incidental crossovers. On the basis of data from the 116 unselected tetrads, 10% (12) of the tetrads contained an incidental crossover; incidental crossovers are defined as those in which the crossover involved is not located at the end of a tract of gene conversion/PMS. The likelihood of an incidental crossover involving the chromatid containing the aberrant segregation events is, therefore, 5% (0.10×0.50). Since the incidental crossover must also occur adjacent to the tract of aberrant segregation to be considered an associated crossover, the likelihood would be reduced to <5%. Given 34 unidirectional events in which the crossover could be unambiguously mapped, <2 would be expected to be scored as containing a crossover configuration of the flanking markers due to incidental crossovers.

Of the eight crossover events that could be mapped to a single interval, five involved the 380-bp interval II, one involved the 4.5-kb interval I, and two involved the 6-kb interval III. On the basis of the relative sizes of these intervals, these results suggest a strong preference for resolution of the unidirectional events as crossovers at a position near the initiating DSB. In a study similar in design to ours, L. JESSOP and M. LICHTEN (personal communication) found that ~85% of the aberrant segregation events were unidirectional, and there was an even stronger bias in favor of crossover resolution near the initiating DSB.

In addition to the 40 tetrads that had a single recombination event consistent with a unidirectional heteroduplex initiated at the *HIS4* hotspot, there were an additional 6 tetrads consistent with a unidirectional heteroduplex initiated at the *HIS4* hotspot plus an incidental exchange (Table IV, classes 87, 88, 110, and 111 and Table V, classes 148 and 149). An example of such a tetrad is shown in Figure 3c. These tetrads are included in Table 2, which summarizes the number of uni- and bidirectional tetrads obtained in JDM1080 and JDM1086 with different methods of analysis. Patterns of the unidirectional events are shown in Figure 4a.

Events were classified as bidirectional if they involved two tracts of aberrant segregation, one continuous tract involving markers on one side of the DSB in one spore and a second continuous tract involving markers on the other side of the DSB in a different spore (Table II, classes 26–36). To classify a tetrad as a bidirectional event, we required that at least one of the markers on each side of the DSB site had undergone a PMS event. This requirement was imposed because coconversion events involving *bik1-lop* and *his4u-lopc* could represent a recombination event initiated at a DSB site other than that located between *bik1-lop* and *his4u-lopc*. This issue is discussed further in a separate section of RESULTS.

The bidirectional events are predicted by the DSB model in Figure 1, but have been observed only very rarely in previous studies (PORTER *et al.* 1993; GILBERTSON and STAHL 1996). About 30% (16 of 56) of the single recombination events initiated at the *HIS4* DSB are bidirectional and 64% (9 of 14 tetrads in which

TABLE 2

Classification of recombination events initiated at *HIS4*

Strain	Method of analysis	Unidirectional events			Bidirectional events		
		NCO	CO	AMB	NCO	CO	AMB
JDM1080	Unselected	5	2	1	0	1	0
JDM1086	Unselected	6	4	2	1	1	1
JDM1086	Selected-1[a]	6	4	4	3	7	1
JDM1086	Selected-2[a]	5	3	0	1	3	1
JDM1086	Strand transfer	4	0	0	1	1	1
Total		26	13	7	6	13	4

NCO, noncrossover; CO, crossover; AMB, ambiguous—event involved conversion of one of the flanking markers, so it was not possible to definitively assign the crossover status of the event.

[a] Selection schemes are described in Table 1.

the configuration of the flanking sequences could be unambiguously assigned) of the bidirectional events are associated with crossovers. Examples of single bidirectional events without and with associated crossovers are shown in Figure 3, d and e, respectively. In 8 of the 9 tetrads (Table II, classes 26, 28, and 31–33), Holliday junction resolution involved cleavage of the strands that the DSBR model (Figure 1) predicts would contain newly synthesized DNA. Similar biases have been observed previously and are explicable as targeted cleavage of the Holliday junctions directed by the nicked strand (reviewed by Foss *et al.* 1999).

In addition to those tetrads shown in Table II, we found seven additional tetrads in which a bidirectional event occurred in a tetrad with an incidental exchange (Table IV, classes 89, 90, and 112–116). These tetrads are included in Table 2 and Figure 4. In tetrads in which crossovers could be unambiguously mapped, 13 of 39 unidirectional events were associated with a crossover, and 13 of 19 bidirectional events were crossover associated. These levels of association are significantly different ($P = 0.02$, Fisher's exact test). If tetrads in which all of the aberrantly segregating markers representing conversion rather than PMS events are excluded from the analysis of the unidirectional events, then 11 of 32 unidirectional events are crossover associated, compared to 13 of 19 bidirectional events ($P = 0.02$, Fisher's exact test).

Since the number of bidirectional events is relatively small, one issue to consider is whether such events could reflect two independent unidirectional events. In 116 unselected tetrads, if we include the bidirectional events as representing two unidirectional events, there were 14 (12%) tetrads with aberrant segregation patterns of *bik1-lop* consistent with a unidirectional event initiated at the *HIS4* hotspot and 14 tetrads with aberrant segregation patterns of *his4u-lopc* consistent with a unidirectional event initiated at the *HIS4* hotspot. The predicted fraction of tetrads in which two such unidirectional events would mimic a bidirectional event is (1/2) (1/2)(0.12)(0.12) or 0.0036. The two factors of one-half reflect the probabilities that both events will be in the same direction (both 5:3/6:2 or 3:5/2:6) and that the events will involve different chromatids. The observed frequency of bidirectional events (23/1603) is at least fourfold higher than this value.

A similar conclusion can be made on the basis of a somewhat different type of argument. In 712 tetrads of JDM1086 examined by the selected-1 method, we found 10 in which the *bik1-lop* and *his4u-lopc* markers both segregated 5:3 or 3:5 in different spores as expected for bidirectional DSBR events. Only 1 tetrad had a 5:3 segregation at *his4u-lopc* and a 3:5 segregation at *bik1-lop* in a different spore, and none had a 3:5 at *his4u-lopc* and 5:3 at *bik1-lop*. Thus, the patterns expected for the bidirectional DSBR events are more common than those expected for two unidirectional events.

On the basis of the orientation of the direction of transcription of *HIS4* and the 5′ to 3′ resection of the broken ends, recombination events initiated by a DSB at the *HIS4* hotspot will involve a heteroduplex in which the nontranscribed strand of *HIS4* is the donor and the transcribed strand (derived from the chromosome that received the DSB) is the recipient (Nag and Petes 1990). This prediction can be tested using a specialized type of tetrad analysis in which the tetrads are dissected onto plates containing medium lacking histidine (details in materials and methods). After ~11 hr, the spores are scored as His^- or His^+. The medium containing the dissected spores is then transferred to plates containing excess histidine, and the histidine diffuses into the medium lacking histidine, allowing nonselective growth of the spores. When spore colonies have formed, they are replica-plated to medium lacking histidine to score His^+/His^- sectored colonies. By correlating the aberrant segregation pattern with the scoring of the spore phenotypes on the histidine omission medium, we can determine for tetrads with a single PMS event involving *his4-IR9* which strand was transferred (Nag and Petes 1990, Figure 2). For example, if the

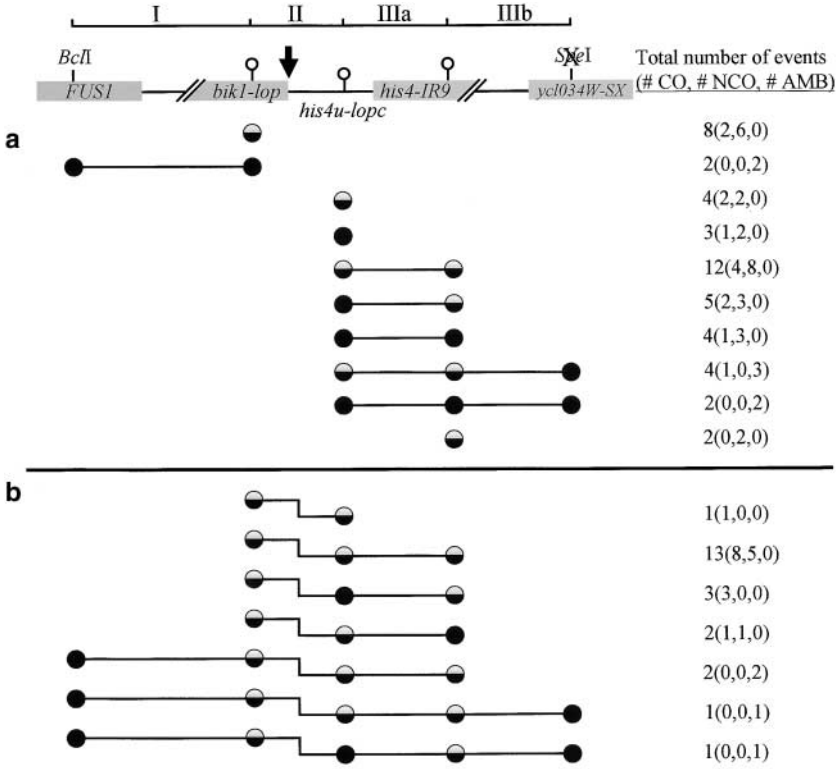

FIGURE 4.—Numbers of tetrads with various patterns of aberrant segregation interpretable as unidirectional (a) and bidirectional (b) events initiated at the DSB associated with the *HIS4* hotspot. In this diagram, solid circles indicate a gene conversion event, and solid/open sectored circles show a PMS event. The unidirectional events involve a single chromatid (circles connected by horizontal lines show aberrant segregation events in the same direction), whereas the bidirectional events involve different chromatids on each side of the DSB (indicated by a short vertical line connecting horizontal lines). In a, the tetrad classes (derived from Tables I–V) used for each line of data (line 1 indicating the top line) are as follows: line 1, classes 1–4; line 2, classes 5 and 87; line 3, classes 6–8 and 110; line 4, classes 9–11; line 5, classes 12–16, 111, 148, and 149; line 6, classes 17, 18, and 88; line 7, classes 19–21; line 8, classes 22 and 23; line 9, class 24; and line 10, class 25. In b, the comparable information is as follows: line 1, class 26; line 2, classes 27–31, 89, and 112–114; line 3, classes 32 and 33; line 4, classes 34 and 115; line 5, classes 35 and 90; line 6, class 116; and line 7, class 36.

sectored colony from a 5^+:3^- segregation event was derived from a His^- spore, we can infer that the donor wild-type strand was nontranscribed. It should be pointed out that any recombination event initiated centromere-proximal to *his4-IR9* that includes this marker in a heteroduplex event will result in donation of the nontranscribed strand (according to the canonical DSBR model), whereas events initiated by a DSB centromere-distal to the marker will result in donation of the transcribed strand.

Strand transfer analysis of a limited number of tetrads confirmed our interpretation of most of the single events initiated between the palindromes. Of six events, three unidirectional and three bidirectional, initially assigned as being initiated by the *HIS4* DSB, all of the bidirectional events and two of the three unidirectional events involved transfer of the nontranscribed strand. The one unidirectional event resulting from the transfer of the transcribed strand involved postmeiotic segregation of *his4-IR9* with coconversion of *his4u-lopc* and *ycl034W-SX*. An alternative interpretation of this event is that it initiated centromere-distal to the *ycl034W-SX* marker and terminated between *bik1-lop* and *his4u-lopc*.

Single recombination events that include markers in the *HIS4* region that are initiated at a site different from the *HIS4* DSB: From previous studies analyzing meiosis-specific DSBs on chromosome III (BAUDAT and NICOLAS 1997; GERTON *et al.* 2000), it is clear that there are a number of strong DSB sites near *HIS4* other than the one located immediately upstream of *HIS4*. We mapped DSBs in the region around *HIS4* in strains JDM1081 and QF105 (details in MATERIALS AND METHODS). The patterns of DSBs were the same in the two strains, indicating that the *bik1-lop* and *his4u-lopc* markers do not affect DSBs. In Figure 5, the sizes of the arrows indicate the approximate strength of the DSBs. Many of the tetrads examined in our study (77 of 1603) have patterns of aberrant segregation consistent with single initiation events at one of the DSBs that is not associated with the *HIS4* hotspot (Table III, classes 37–86; Figure 3f). We define this type of tetrad as (1) those that have aberrant segregation for *ycl034W-SX*, *fus1-BX*, or *his4-IR9*, but not for either *bik1-lop* or *his4u-lopc* and (2) those with coevents that span the *bik1-lop* and *his4u-lopc* markers. One particularly interesting type of tetrad (Figure 5; Table III, classes 50–52) represents coconversion events that include all markers in the 10.5-kb region that includes *HIS4*; this type of tetrad is discussed in a separate section. Figure 5 also includes an additional 7 tetrads that were interpretable as events initiated at a site different from the *HIS4* hotspot and that had an incidental exchange (Table IV, classes 91–93 and 117; Table V, classes 150–152).

Support for these classifications was provided by strand transfer experiments. Of 11 events classified as initiating from a DSB other than the *HIS4* hotspot, 4 were events in which the transcribed strand of *HIS4* was donated. As discussed above, such events are likely to reflect DSBs that are centromere-distal to the *HIS4* hotspot DSB. Most of the other events are likely to represent events initiated from centromere-proximal DSBs. Two tetrads, however, yielded an unexpected pattern. In

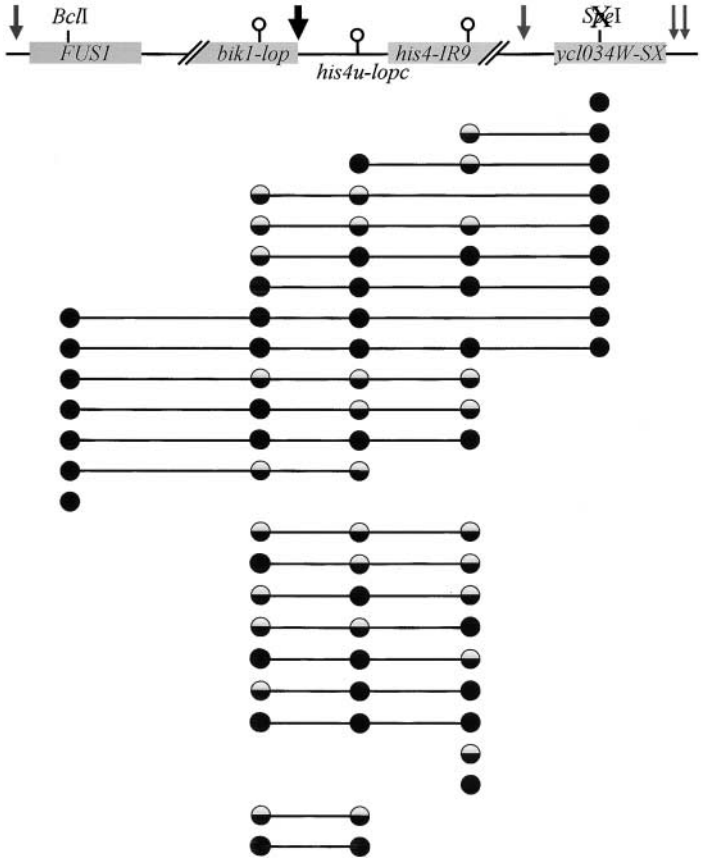

FIGURE 5.—Summary of aberrant segregation events interpretable as initiating at sites other than the *HIS4* hotspot-associated DSB in JDM1080 and JDM1086 tetrads. Arrows show the position and intensity of DSBs in this region. The *HIS4* hotspot-associated DSB (occurring near the 3′ end of *BIK1*) represents ~5% of the DNA molecules. Normalizing this DSB to a value of 1, the other DSBs had approximate intensities of 0.5 (*FUS1*-associated DSB), 0.5 (DSB near *YCL034W* on *HIS4* side), and 0.25 (doublet on other side of *YCL034W*). The horizontal lines show the extent of coevents (continuous tracts of conversion and/or PMS). Solid circles indicate conversion and sectored circles indicate PMS. In all cases, the events were in the same direction. The tetrad class marked with an asterisk shows a pattern of aberrant segregation that would be consistent with a unidirectional event initiated at the *HIS4* hotspot, except for the observation that it involved transfer of the transcribed strand of *HIS4*.

these two tetrads, the *his4-IR9* marker, but not the *his4u-lopc* marker, showed aberrant segregation. We expected these two tetrads to reflect a DSB located centromere-distal to the *HIS4* hotspot and, therefore, to involve donation of the transcribed strand. Both, however, involved transfer of the nontranscribed strand. The segregation patterns of these tetrads could be generated by a DSB at the *HIS4* hotspot, heteroduplex formation that includes both *his4u-lopc* and *his4-IR9*, and with restoration repair of the *his4u-lopc* mismatch. Alternatively, these patterns could reflect recombination initiated by a DSB between *his4u-lopc* and *his4-IR9*.

We also found one tetrad (class 43, Table III) that was a coevent involving the *his4u-lopc*, *his4-IR9*, and *ycl034W-SX* markers, similar to one class of unidirectional events shown in Table I. This tetrad was not considered a unidirectional event initiated at the *HIS4* hotspot, however, because a strand transfer experiment indicated that the donated strand was the transcribed strand of *HIS4*.

In summary, we conclude that ~56% of the recombination events that involve *his4-IR9* initiate at DSBs at sites different from the *HIS4* hotspot. Our data demonstrate that the recombination activity at a specific site in the genome represents the integration of recombination activities initiated at multiple DSB sites. This conclusion, although somewhat surprising, is consistent with our previous observations that mutational changes (for example, elimination of the Rap1p binding site in the *HIS4* promoter) that block DSB formation at *HIS4* reduce aberrant segregation of *his4-IR9* by only twofold (FAN *et al.* 1995).

At many loci, the frequency of gene conversion of a mutant site is a linear function of its position within the gene (reviewed by NICOLAS and PETES 1994). Such gradients of gene conversion are termed "polarity gradients." At both the *ARG4* and *HIS4* loci, the frequency of conversion declines from the 5′ end to the 3′ end, although the extent of this decline is quite different. At the *ARG4* locus, the rates of conversion differ by about a factor of 10, whereas at the *HIS4* locus, the difference is only a factor of 2.5 (NICOLAS and PETES 1994). Several types of mechanisms have been suggested to contribute to the formation of polarity gradients: (1) the extent of resection of DNA from the DSB site (SUN *et al.* 1991), (2) a distance-dependent alteration in the ratio of conversion-type to restoration-type repair (DETLOFF *et al.* 1992) perhaps directed by the resolution of Holliday junctions (FOSS *et al.* 1999), and (3) distance-dependent abortion of heteroduplexes directed by the mismatch repair system (ALANI *et al.* 1994). Our results argue that the shape of the polarity gradient may also reflect differential contributions of heteroduplexes initiated at multiple DSB sites.

Meiotic recombination events that may reflect break-induced replication or gap repair: In 9 of 1603 tetrads

(Figure 5), all five markers underwent conversion, either all 6:2 or all 2:6 (Table III, classes 50–52). These events are unusual in two ways. First, the conversion tracts were unusually long, minimally 10.5 kb. Second, the palindromic markers, which usually exhibited postmeiotic segregation, instead underwent conversion. In 9 of 10 tetrads in our study in which the flanking *fus1-BX* and *ycl034W-SX* markers coconverted, the intervening palindromic insertions also coconverted. In 20 of 22 tetrads in which the palindromic insertions, but not the flanking *fus1-BX* and *ycl034W-SX* markers, underwent coaberrant segregation (Table III, classes 64–78; Table IV, classes 92 and 93), one or more of the palindromic insertions had a PMS event. This difference is very significant ($P = 0.0001$).

One interpretation of this result is that such tetrads reflect a very long heteroduplex that covers all five markers. Excision tracts extending from the mismatches involving the *fus1-BX* and *ycl034W-SX* markers could result in the corepair of the mismatches resulting from the palindromic insertions. This interpretation is unlikely, however, since two-thirds of meiotic excision repair tracts are <1 kb, and none >1.8 kb were detected (Detloff and Petes 1992). In addition, of 22 tetrads (Table III, classes 41–49, 53–60, and 91) that include either *fus1-BX* or *ycl034W-SX* (but not both markers) and one or more of the palindromic sites in a coaberrant segregation event, 16 had PMS events at one or more of the palindromic insertions.

We favor the alternative possibility that the class 50–52 tetrads are gene conversion events that do not involve heteroduplex formation followed by DNA mismatch repair. We suggest two possibilities. The first is that these conversion events reflect meiotic break-induced replication (BIR) events. In BIR events, which have been invoked as a model to explain very long mitotic gene conversion tracts (reviewed by Paques and Haber 1999), a broken end of one chromosome invades a second, setting up a unidirectional replication process that proceeds to the end of the chromosome (Figure 6a). A second possibility is that the class 50–52 tetrads are a consequence of a chromatid with two closely spaced DSBs. If the broken ends derived from different DSBs are used to set up the double Holliday junctions with loss of the DNA fragment between the breaks, a gene conversion event that does not involve DNA mismatch repair would occur (Figure 6b). This model is essentially that proposed originally for the DSBR model (Szostak *et al.* 1983) except that the gap is a consequence of two DSBs rather than a single DSB that is processed by degradation of both DNA strands.

The *HIS4* gene is ~67 kb from the left telomere of chromosome III. We constructed two strains that were isogenic with JDM1086 except for the inclusion of a heterozygous insertion of the HYG^R gene into *CHA1*, a gene located ~16 kb from the telomere. In strains MD250 and MD251, the insertions were on the opposite

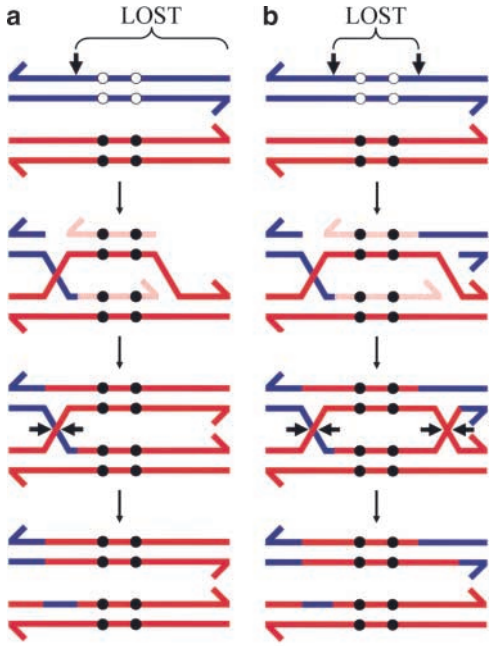

Figure 6.—Mechanisms leading to coconversion without mismatch repair within a heteroduplex. (a) BIR. In this mechanism (reviewed by Paques and Haber 1999), one broken end invades a chromosome and replication proceeds to the end of the intact DNA molecule. Subsequently, the resulting junction is cleaved. (b) Gap repair. One chromatid receives two DSBs, with loss of the DNA fragment located between the two DSB sites. The resulting gap is filled in by repair synthesis, and the junctions are cleaved. Although we show the cleavage pattern that would generate a noncrossover configuration of flanking markers, the intermediate could also be resolved to generate a crossover.

homolog or the same homolog, respectively, as the palindromic insertions. In all tetrads derived from these diploids, we scored segregation of the *his4-IR9* and the *cha1::hphMX4* markers. In those tetrads in which the *his4-IR9* marker underwent gene conversion, we examined the segregation of the palindromic insertions and the flanking markers. The data from this experiment are shown in Table 1.

Of 1429 total tetrads, we found 6 in which all five markers in the *HIS4* region were coconverted (frequency of 0.4%). The *cha1::hphMX4* marker underwent gene conversion in 3 of these tetrads (all derived from MD251) and segregated 2:2 in 3. Although this number of these tetrads is low, since the rate of aberrant segregation of the *cha1::hphMX4* marker is only 2.1% (Table 1), it is statistically significant ($P = 0.0002$); in all such tetrads, four other heterozygous markers segregated 2:2, indicating that these exceptional tetrads are not likely to be false. Of these 3 tetrads, however, only 1 had the pattern shown in Figures 6a and 7a. In this tetrad, three spores were *fus1-BX BIK1 HIS4U HIS4 YCL034W CHA1* and one was *FUS1 bik1-lop his4u-lopc his4-IR9 ycl034W-SX cha1::hphMX4*, as expected for a single BIR event. In a second tetrad, one spore was *fus1-BX BIK1 HIS4U HIS4*

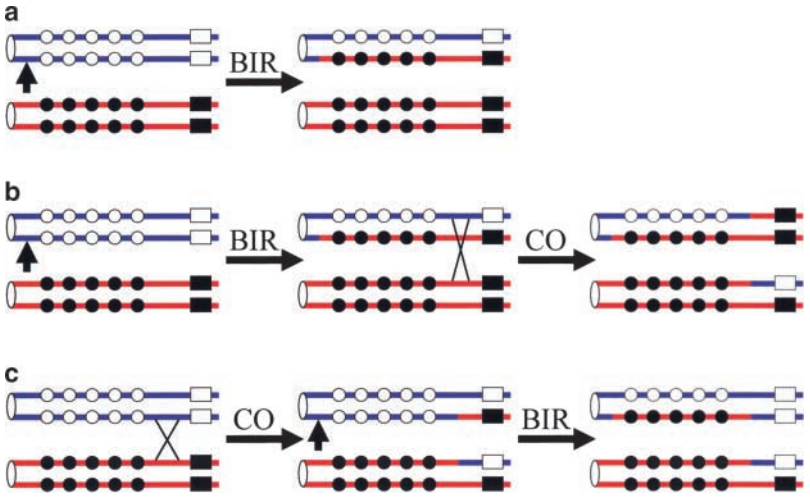

FIGURE 7.—Expected patterns of aberrant segregation after BIR alone and BIR with a crossover. Open and solid circles indicate the five heterozygous markers in the *HIS4* region and the open and solid rectangles indicate the presence or absence of the *cha1::hphMX4* allele. The *cha1::hphMX4* marker is ~50 kb from the markers in the *HIS4* region. The arrow shows the position of the initiating DSB, and we assume that the centromere-distal fragment is lost. (a) Pattern expected for BIR without a crossover between the *HIS4* markers and the *cha1::hphMX4* marker. (b) Pattern expected for a BIR event and single crossover between the *HIS4* markers and *cha1::hphMX4* after the completion of BIR. (c) Pattern expected for a crossover between the *HIS4* markers and *cha1::hphMX4* before BIR.

YCL034W cha1::hphMX4, two were *FUS1 bik1-lop his4u-lopc his4-IR9 ycl034W-SX cha1::hphMX4*, and one was *FUS1 bik1-lop his4u-lopc his4-IR9 ycl034W-SX CHA1*. The pattern of segregation observed in this tetrad is consistent with a single BIR event, followed by a crossover between the *YCL034W* and *CHA1* genes (Figure 7b). In the third tetrad, we found one spore was *fus1-BX BIK1 HIS4U HIS4 YCL034W CHA1*, two were *FUS1 bik1-lop his4u-lopc his4-IR9 ycl034W-SX CHA1*, and one was *FUS1 bik1-lop his4u-lopc his4-IR9 ycl034W-SX cha1::hphMX4*. This pattern of segregation can be explained by a crossover between the *YCL034W* and *CHA1* genes that preceded a BIR event (Figure 7c). It should be pointed out that all 3 of the diagnostic tetrads can also be explained as gap repair events in which one DSB occurs centromere-proximal to the markers in the *HIS4* region and the other occurs centromere-distal to the *cha1::hphMX4* marker. Because of the limited distance between the *cha1::hphMX4* marker and the telomere, we prefer the hypothesis that they represent BIR events.

In three of the six tetrads with coconversion of markers in the *HIS4* region, the *cha1::hphMX4* marker segregated 2:2. We suggest that these events represent the repair of a double-strand gap, as discussed above (Figure 6b). We cannot exclude the possibility that these events reflect very long heteroduplexes in which the resulting DNA mismatches are repaired by a process involving very long excision repair tracts, although no experimental evidence supports such a mechanism.

Tetrads with unambiguous multiple recombination initiation events: Although the classification of tetrads as representing single or multiple events depends, to some extent, on what assumptions are allowed concerning heteroduplex formation (symmetric or asymmetric) and the patterns of DNA mismatch repair, tetrads with more than two recombinant chromatids (for example, Figure 3c) or that have markers on the same chromatid that segregate in opposite directions (for example, 5:3 for *bik1-lop* and 3:5 for *his4u-lopc*) must represent multiple initiation events (Table IV). We also include in this table tetrads that have two recombinant chromatids and two chromatids with one parental configuration of markers, but no chromatids with the other parental configuration. A total of 50 tetrads involving these classes of multiple events were observed (Table IV). In 23 tetrads, three chromatids were recombinant (classes 87–109); in 13, four were recombinant (classes 110–122); 5 tetrads involved either three or four chromatids (classes 123–127). We expect an ~2:1 ratio of three-chromatid to four-chromatid events for double recombination events, since there are two ways of involving three chromatids, but only one way of involving four.

In addition to the 41 tetrads that have involvement of more than two chromatids in 1 tetrad, there were 9 tetrads with two recombinant chromatids, but markers that segregated in opposite directions. These tetrads represent classes 128–136 in Table IV. It should be pointed out that when tetrads could be classified as a multiple event by more than one criterion, we assigned them into one of the classes arbitrarily.

Tetrads that represent either single or multiple recombination initiation events, depending on the assumptions about the mechanism of recombination: Thirty-four tetrads could be classified as representing either single or multiple recombination events (Table V). All tetrads in this category had no more than two recombinant chromatids and, if more than one marker underwent aberrant segregation, the markers segregated in the same direction. The different types of tetrads in this group included: (1) tetrads in which the continuity of a conversion/PMS tract was disrupted by a marker that undergoes Mendelian segregation (classes 137–147), (2) tetrads in which the crossover was separated from the aberrant segregation tract by at least one other marker that undergoes Mendelian segregation (classes 148–152), (3) tetrads that had spores with two PMS events in which the palindromes were in different DNA strands (*trans* events; classes 153–160), (4) tetrads with more than one PMS event for a single marker (classes 161–164), and (5) tetrads with a crossover be-

tween two markers showing aberrant segregation in the same direction (classes 165–167). Although all of these tetrads can be explained as representing multiple initiation events, all are also consistent with events initiated by a single DSB, as described below.

Noncontiguous tracts of aberrant segregation (for example, one marker segregating 2:2 with flanking markers segregating 5:3) can be explained as two DSBs giving rise to two heteroduplex regions or as a single heteroduplex in which the middle marker undergoes restoration-type repair; this type of repair of mismatches in heteroduplexes results in Mendelian segregation instead of gene conversion (conversion-type repair) or PMS (failure to repair). Although restoration-type repair events near the site of the DSB are infrequent (Detloff *et al.* 1992), such events occur at mismatches that are displaced from the initiating lesion (Kirkpatrick *et al.* 1998). Restoration-type repair can also explain tetrads in which the crossover is separated from the tract of gene conversion/PMS by a marker exhibiting Mendelian segregation.

We found many tetrads in which more than one marker underwent PMS in the same direction (both 5:3 or both 3:5). For most such tetrads, we determined whether the event involved transfer of the same DNA strand (as described in materials and methods); this analysis was done on all of the unselected tetrads and tetrads examined by the strand transfer method of analysis and more than half of the tetrads examined by the S-1 method. Although most of these co-PMS events involved transfer of the same DNA strand (*cis*), we found 12 tetrads in which the palindromic insertions were in different strands (*trans*); 2 of these tetrads were classified as representing double events for other reasons (classes 94 and 95, Table IV), whereas 10 were classified as double events solely as a consequence of the *trans* configuration (classes 153–160, Table V). Such *trans* events were found previously (Porter *et al.* 1993; Gilbertson and Stahl 1996). The *trans* events could represent a double SDSA event. In such double events, we suggest that one end invades and is used as a primer for DNA synthesis. The invading strand is then displaced. The second end then invades, is used as a primer for DNA synthesis, and is removed. Alternatively, these events could represent single events initiated at the *HIS4* hotspot in which only one Holliday junction is cut and the other junction branch migrates (Figure 5 in Gilbertson and Stahl 1996). Four of the 10 tetrads with the *trans* configuration had an associated crossover, indicating that whatever intermediates are involved in producing the *trans* configuration, they must have the option of resolution as a crossover.

In some tetrads, one or more markers exhibited aberrant 4:4 segregation (one wild-type spore colony, one mutant spore colony, and two sectored spore colonies). This pattern of segregation can be a consequence of formation of symmetric heteroduplexes, in which a single initiating event generates heteroduplexes at the same site on two different chromatids (Holliday 1964), or can reflect two independent initiation events, each involving asymmetric formation of heteroduplexes. Since the frequency of aberrant 4:4 tetrads in *S. cerevisiae* is roughly that expected for two independent events, it has been argued that symmetric heteroduplex formation is infrequent (Fogel *et al.* 1981; Petes *et al.* 1991). This conclusion was supported by investigations of the frequency of aberrant 4:4 tetrads in strains with different levels of hotspot activity at the *HIS4* locus (Fan *et al.* 1995). Gilbertson and Stahl (1996) and Stahl and Hillers (2000), however, pointed out that the mechanism by which a recombination intermediate was resolved would influence the ability to detect symmetric heteroduplexes. For example, a symmetric heteroduplex intermediate that was resolved by topoisomerase, rather than cleavage of Holliday junctions, would not be detectable as an aberrant 4:4 segregation. In addition, Hillers and Stahl (1999) found that some classes of aberrant 4:4 tetrads had patterns of associated crossovers consistent with symmetric heteroduplex formation, whereas others did not. In our view, the most likely interpretation of the existing data is that most observable aberrant 4:4 segregation events represent two initiation events associated with asymmetric heteroduplexes, although some reflect symmetric heteroduplexes. It should be pointed out that, of the 17 tetrads with markers that had double PMS or double gene conversion events, 13 were tetrads in which there were more than two recombinant chromosomes, strongly suggesting that they represent double initiation events.

We also classified tetrads in which a crossover occurred within a tract of aberrant segregation as representing ambiguous multiple events. Such events could also be explained as a single recombination intermediate in which branch migration moved the Holliday junction into the tract of aberrant segregation (following DNA mismatch repair). On the basis of the low frequency of aberrant 4:4 tetrads, which argues against extensive branch migration (Fogel *et al.* 1981), we expect this class to be infrequent.

Although the tetrads depicted in Table V could represent either single or double events, it is likely that the majority of these tetrads represent single initiation events. This conclusion is based on the fraction of these tetrads in which there is involvement of two, three, or four chromatids. Assuming that there is no positive or negative chromatid interference for the initiation of recombination events, one would expect that double events would involve two, three, or four chromatids in an approximate ratio of 1:2:1. Thus, three-quarters of the double events would be expected to be three- or four-chromatid events. If we consider all 84 tetrads in Tables IV and V, we find that 41 represent three- and four-chromatid events, and 43 are two-chromatid events. The simplest way of explaining the excess of two-chro-

matid events is that many of the ambiguous "multiple" events in Table V represent single initiations.

Because of the ambiguities involved in the interpretation of these tetrads and others that may represent multiple initiation events, our discussion of models of recombination emphasizes those tetrads that can be easily explained as resulting from a single initiating DNA lesion.

Crossovers unassociated with aberrant segregation: Although most of our analysis was done with tetrads that were screened for aberrant segregation of *his4-IR9*, we also nonselectively examined 116 tetrads. Seven of these tetrads had crossovers without aberrant segregation of any of the five markers in the *HIS4* region. In tetrads derived from JDM1086, we found 2, 1, and 1 tetrad with crossovers in regions I, II, and IIIb, respectively. We also found 3 tetrads in strain JDM1080 with a crossover in the interval IIIa/IIIb.

DISCUSSION

Our results, as well as those of others, indicate the difficulty and, perhaps, the futility of explaining all meiotic recombination activities on the basis of a single model. At the *HIS4* recombination hotspot, we suggest that there are at least three types of recombination events, all initiated by DSBs: (1) events that occur through the canonical DSBR pathway, (2) SDSA events, and (3) BIR and/or gap repair events. Each of these classes is discussed separately below.

Canonical DSBR pathway of recombination: Some events represent formation and resolution of double Holliday junctions as predicted by a slightly modified form of the canonical DSBR model shown in Figure 1. Since very few of these events were observed in our previous study in which the flanking markers were 700–1000 bp from the DSB site (Porter *et al.* 1993), we suggest that the initial strand invasion produces a region of heteroduplex that is variable, but often <200 bp. Since preliminary studies indicate that the resection of the DSB at the *HIS4* hotspot is usually >400 bases, we argue that the size of the heteroduplex is not determined solely by the extent of resection. The heteroduplex resulting from primed synthesis of the invading strand results in a second, and much more extensive, region of heteroduplex (Figure 8). We previously proposed that the unidirectional events resulted from asymmetric resection of the DNA ends produced by the DSB (Porter *et al.* 1993). Although we cannot exclude this model, on the basis of preliminary observations of symmetrically resected ends at the *HIS4* hotspot (data not shown), we suggest that the extent of heteroduplex is not directly related to the extent of resection.

The observed asymmetry in heteroduplexes flanking the DSB site can also be explained by other versions of the DSBR model. For example, it is possible that an extensive heteroduplex region is formed by the strand

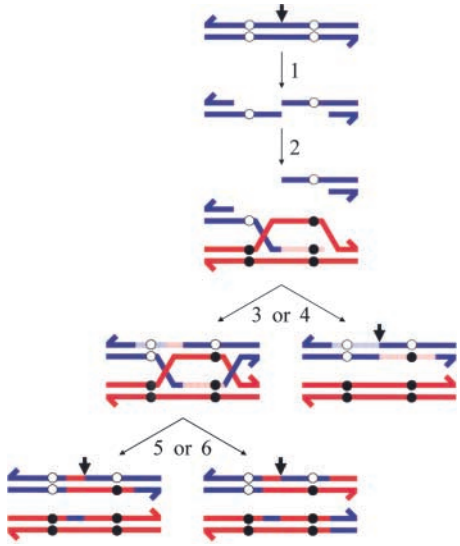

Figure 8.—Two mechanisms that generate the unidirectional recombination events observed at the *HIS4* hotspot. We suggest that the unidirectional events have two sources. Both types of events involve the same early steps: DSB formation, followed by DSB processing (step 1), and single-ended invasion, followed by primed DNA synthesis (step 2). On the left part of the diagram, the second broken end forms a heteroduplex (step 3), and the resulting intermediate is processed to yield either a noncrossover (step 5) or a crossover (step 6). On the right part of the diagram, the single-ended invasion is reversed without crossing over (step 4).

invasion, and the limited heteroduplex region results from limited DNA synthesis primed by the invading strand. Although we cannot rule out this model, we favor the first model for two reasons: (1) physical data argue that *HIS4* DSBs are resected by ~600 bp (Nag and Petes 1993; our unpublished data), an amount too limited to account for the very long (2.7 kb) heteroduplexes observed at *HIS4* (Detloff *et al.* 1992), and (2) large (>1 kb) heterozygous insertions within the *HIS4* gene are readily incorporated into heteroduplexes, an observation more compatible with DNA replication-driven heteroduplex formation than with passive DNA strand invasion or DNA branch migration.

Allers and Lichten (2001b) physically demonstrated the existence of DNA molecules with heteroduplexes flanked by double Holliday junctions ("JM1" intermediates) as predicted by the DSBR model. In addition, they observed a recombination intermediate in which the double Holliday junctions were located on one side of the DSB, and the marker at the DSB site was not in a heteroduplex ("JM2" intermediates). They explained this intermediate by a model [the strand-displacement model; Figure 4c of Allers and Lichten (2001b)] in which strand invasion and DNA synthesis occur on only one side of the DSB. The invading strand is partially displaced and pairs with the other end of the broken chromosome. The net result of these events is that the double Holliday junction is located to one

side of the DSB and no heteroduplex is observed on that side of the DSB. There is, however, a region of heteroduplex on the opposite side of the DSB. Although we found a few tetrads with the segregation pattern expected for this type of event (classes 148 and 149), such tetrads were rare. Many of the crossovers observed in our experiments were located downstream of the marker showing aberrant segregation, as expected from the canonical DSBR model. In addition, many crossovers occurred near the DSB site. This class can be explained by the standard DSBR model, assuming one of the heteroduplexes is very small. Alternatively, this class is consistent with the strand-displacement model if the region of DNA that is displaced is very small.

Our analysis almost certainly underestimates the frequency of bidirectional events for two reasons. First, we classified tetrads as bidirectional only if at least one marker on each side of the *HIS4* DSB site underwent PMS in the same direction. Tetrads that had conversion on one side of the DSB site and PMS on the other (for example, Table III, class 68) or conversion on both sides of the DSB site (for example, Table III, class 78), which were usually classified as recombination events initiated at sites other than the *HIS4* hotspot, could represent bidirectional events initiated at the *HIS4* hotspot. Since we cannot unambiguously identify the spore involved in heteroduplex formation at a site that manifests gene conversion, we chose the most conservative interpretation of the tetrads. Second, the patterns of aberrant segregation of some tetrads (for example, Table V, class 166) are consistent with bidirectional events initiated at sites other than the *HIS4* hotspot.

One issue that is still unclear is why we detected bidirectional events at the *HIS4* hotspot, and a similar study, using markers placed at similar distances from the *ARG4* hotspot, found such events very rarely (4 in 4147 tetrads; Gilbertson and Stahl 1996). The obvious possibility is that the ratio of various types of recombination events varies in a locus- and/or strain-dependent manner. One relevant difference may be the extent of heteroduplex formation. At the *HIS4* hotspot, heteroduplexes often extend >2.5 kb from the initiating DSB (Detloff *et al.* 1992); at the *ARG4* locus, heteroduplexes usually extend <1 kb (Sun *et al.* 1991). As discussed below, mismatch repair events directed by nicks formed during resolution of the Holliday junction may result in restoration-type repair of mismatches (Alani *et al.* 1994; Foss *et al.* 1999). If the efficiency of this process is related to the distance between the mismatch and the nick, bidirectional events would be easier to detect at the *HIS4* hotspot than at the *ARG4* hotspot.

SDSA events: A substantial fraction of the recombination events were unidirectional, involving only one of the flanking markers. Some of these events are likely to resemble that shown in Figure 8, but in which the heteroduplex formed by strand invasion did not include the flanking marker. Since the unidirectional events were significantly less associated with crossing over than were the bidirectional events (one-third and two-thirds, respectively), it is likely that some of these events reflect SDSA (Allers and Lichten 2001a,b). If none of the SDSA events are associated with crossovers, we calculate that about one-half of the unidirectional events represent SDSA events. In addition, we observed nine tetrads in which one spore had PMS events for the palindromic markers flanking the *HIS4* DSB site and in which different strands were involved in heteroduplex formation (*trans* events). As described previously, such tetrads could result from two consecutive SDSA events, one involving each DNA end, although other interpretations are also possible.

An alternative explanation of the observation that unidirectional events are less frequently associated with crossovers than are bidirectional events is that DSBR intermediates with long heteroduplexes (detected as bidirectional events) are more likely to be resolved as crossovers than are DSBR intermediates in which at least one of the heteroduplexes is short (detected as unidirectional events). Although we cannot rule out this model, we prefer the interpretation that some of the unidirectional events reflect SDSA, since there is no obvious mechanism that would restrict SDSA to ectopic exchanges.

A third explanation of unidirectional events has been recently presented by Foss *et al.* (1999). In this model, most recombination proceeds by the canonical DSBR intermediate (heteroduplexes in different chromatids on both sides of the DSB site). Foss *et al.* suggested that efficiently repaired mismatches located near the DSB site are repaired "early," directed by the nick present in the recombination intermediate (leading to conversion). Efficiently repaired mismatches located far from the DSB site are repaired "late," directed by a nick associated with resolution of Holliday junctions, leading to restoration of Mendelian segregation. They suggested that this type of mechanism explains the observation that markers located near the DSB site at *HIS4* preferentially undergo conversion-type repair rather than restoration-type repair (Detloff *et al.* 1992). Foss *et al.* further suggested that markers (such as small palindromic insertions) that result in inefficiently repaired mismatches are not corrected by early mismatch repair (MMR). If the mismatches are located on opposite sides of the DSB, one of the two mismatches will be corrected by late MMR, leading to a tetrad with a unidirectional event.

Although the resolution-directed repair events represent a straightforward explanation of the *HIS4* polarity gradient, in our view, this model is a less satisfactory explanation of the unidirectional events for several reasons. First, the model predicts that markers that lead to inefficiently repaired mismatches and that are located near the initiating DSB will undergo restoration-type repair. The frequency of aberrant segregation of such

markers should be elevated in strains with MMR defects. Nag and Kurst (1997) found that elimination of MMR had little effect on the aberrant segregation frequency of a palindromic marker located near the *HIS4* DSB site. Second, the model proposed by Foss *et al.* requires that mismatches resulting from palindromic insertions be immune to correction early, but susceptible to correction late. In *in vitro* studies, mismatches involving palindromic insertions appear to uncouple DNA binding of the Msh2p-Msh6p complex from the ATPase activity required for subsequent steps in MMR (Alani 1996). Thus, one would expect that these mismatches would be immune to any correction by the canonical MMR system. Third, our finding that bidirectional events at the *HIS4* locus are more frequently observed when the markers are located very near the site of the DSB argues that some of the unidirectional events reflect the small size of the heteroduplex formed by the invading strand. Although none of these arguments is conclusive, we suggest that restoration repair contributes to unidirectional events less than the other mechanisms discussed above (small regions of heteroduplex to one side of the DSB and SDSA events).

BIR/gap repair events: For both the uni- and bidirectional recombination events discussed above, gene conversion reflects the repair of mismatches in heteroduplexes. From our findings of tetrads with concerted repair of mismatches that are usually inefficiently repaired, we suggest that a minority of gene conversion events are not a consequence of the repair of mismatches within a heteroduplex, but reflect either BIR events or repair of a gap resulting from two adjacent DSBs. Such events, although rare, may help explain why mutations in genes involved in DNA mismatch repair reduce, but do not eliminate gene conversion. In addition, our conclusions are consistent with previous observations of continuous gene conversion tracts extending >12 kb (Symington and Petes 1988).

One puzzle is how tightly paired chromosomes in the synaptonemal complex could engage in BIR. It is possible that these events occur after meiotic DNA synthesis, but before chromosome pairing. Alternatively (or in addition), the events could be initiated at the same time as "normal" recombination, but resolved by DNA synthesis after dissolution of the synaptonemal complex.

Multiple recombination events: In addition to the multiple pathways for recombination, our analysis demonstrates that multiple initiation events contribute to the recombination activity of the *HIS4* locus. Some of these events appear to result from multiple initiations at the *HIS4* hotspot, whereas others have the patterns expected for initiations at different DSB sites in the *HIS4* region. As discussed above, these observations demonstrate that the recombination activity of a specific genomic site will be affected by regional, as well as local, hotspot activity. This conclusion is consistent with the observation that the recombination activity of insertions is affected by chromosome context (Borde *et al.* 1999); this effect, in part, is related to regional base composition (Petes and Merker 2002).

Conclusions: Our results, and those of others, suggest that there are multiple pathways of meiotic recombination. The initiating step is likely to be the same for all pathways, an invasion of one chromatid by a processed end derived from the second chromatid. Physical evidence for this intermediate exists (Hunter and Kleckner 2001). Our results suggest that the region of heteroduplex associated with the initial invasion is often limited (250 bp or less), and extensive heteroduplex formation requires DNA synthesis primed from the invading strand (Figure 8). Following DNA synthesis, in about two-thirds of the tetrads, the canonical DSBR intermediate is formed with heteroduplexes on both sides of the DSB site. Most, but not all of these intermediates, are resolved as crossovers. In most of the tetrads in which the second end is not "captured," the heteroduplex intermediate is reversed and rejoined to the other DNA end. In a small subset of the events, however, the invaded end is used as a primer to replicate to the terminus of the chromosome. We also suggest that two DSBs occurring on the same chromatid will sometimes result in a gap repair type of gene conversion.

We thank P. Greenwell for assistance with the Southern analysis and M. Lichten, L. Jessop, H. M. Kearney, F. Stahl, and H. Foss for useful comments on the manuscript and/or communicating unpublished data. The research was supported by National Institutes of Health grant GM-24110.

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Communicating editor: L. S. Symington